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Authors
Shaikh, Kashif
Kinninger, April
Cherukuri, Lavanya
et al.

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Aged garlic extract reduces low attenuation plaque in coronary arteries of patients with diabetes: A randomized, double-blind, placebo-controlled study

KASHIF SHAIKH, APRIL KINNINGER, LAVANYA CHERUKURI, DIVYA BIRUDARAJU, RINE NAKANISHI, SHONE ALMEIDA, ERANTHI JAYAWARDENA, CHANDANA SHEKAR, FERDINAND FLORES, SAJAD HAMAL, MOHAMMED SALMAN SHEIKH, AMIT JOHANIS, BENEDICT CU and MATTHEW J. BUDOFF

Lundquist Institute for Biomedical Innovation at Harbor UCLA Medical Center, Torrance, CA 90502, USA

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Abstract. Several previous studies have demonstrated that aged garlic extract (AGE) inhibits the progression of coronary artery calcification and non-calcified plaque (NCP) in the general population. However, its effects on plaque progression in patients with diabetes have not yet been investigated, at least to the best of our knowledge. This study investigated whether AGE reduces the coronary plaque volume measured by cardiac computed tomography angiography (CCTA) in patients with diabetes mellitus (DM). A total of 80 participants with DM with a median age of 57 years were prospectively assigned to consume 2,400 mg AGE/day (after completion, 37 participants) or placebo (after completion, 29 participants) orally. Both groups underwent CCTA at baseline and follow-up 365 days apart. In total, 66 participants completed the study. Coronary plaque volume, including total plaque (TP), dense calcium (DC), fibrous, fibro-fatty and low-attenuation plaque (LAP) volumes were measured based upon pre-defined intensity cut-off values using semi-automated software (QAngio CT). Changes in various plaque types were normalized to the total coronary artery length. The non-parametric Wilcoxon rank-sum test was performed to examine the differences in plaque formation between the 2 groups. No significant differences were found in the baseline characteristics between the AGE and placebo groups. Compared with the placebo group, the AGE group exhibited a statistically significant regression in normalized LAP [median and standard deviation (SD) -0.2 (18.8) vs. 2.5 (69.3), P=0.0415]. No differences were observed in TP, fibrous, or fibrofatty plaque volumes between the AGE and placebo group. On the whole, this study indicated that the %LAP change in the AGE group was significantly greater than that in the placebo group in patients with diabetes. However, further studies are warranted to evaluate whether AGE has the ability to stabilize vulnerable plaque and decrease adverse cardiovascular events.

Correspondence to: Professor Matthew J. Budoff, Lundquist Institute for Biomedical Innovation at Harbor UCLA Medical Center, 1124 West Carson Street, Torrance, CA 90502, USA
E-mail: mbudoff@labiomed.org

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Introduction

Coronary artery disease (CAD) is the leading cause of morbidity and mortality in individuals with diabetes. According to the report of the American Diabetes Association (ADA), the estimated cost related to the treatment of cardiovascular event is $37.3 billion annually in individuals with diabetes in the US alone (1). Unfortunately, however, the incidence of diabetes is increasing in epidemic proportions worldwide. It is estimated 170 million individuals are living with diabetes worldwide and its prevalence is estimated to double by 2030 (2). A previous study demonstrated that the risk of mortality from cardiovascular causes in subjects with type 2 diabetes mellitus (T2DM) and no previous history of cardiovascular events is equivalent to that of non-DM subjects with prior myocardial infarction (MI) (3). Asymptomatic atherosclerosis has usually been present for years and even decades prior to the development of clinical evidence of CAD (4,5). Screening for the early detection of patients who are at risk of developing asymptomatic atherosclerosis and monitoring their response to treatment in order to reduce cardiovascular events remains a major challenge in preventive cardiology. Coronary computed tomography angiography (CCTA) is considered a reliable tool with which to detect or rule out the presence, extent and severity of coronary atherosclerosis (6).

Previous studies demonstrated that statin and anti-diabetic therapies may decrease the rate of coronary plaque progression and may participate in plaque stabilization (7-9). Previous experiments performed in our laboratory have shown that the use of aged garlic extract (AGE) is associated with a significant reduction in low attenuation plaque (LAP) progression as compared to the placebo using CCTA in patients with metabolic syndrome (10). Furthermore, AGE has been shown to exert positive effects on several cardiovascular disease risk factors, including endothelial dysfunction, cholesterol and blood pressure (11-14). The aim of the present study was to evaluate the effects of AGE over a period of 1 year on coronary atherosclerotic plaque in individuals with T2DM utilizing CCTA.

Patients and methods

Study design. This study was a single-center, randomized, placebo-controlled, double-blind trial being conducted at the Lundquist Institute for Biomedical Innovation at Harbor UCLA Medical Center. In total, 80 individuals, who met the eligibility criteria (inclusion and exclusion criteria) were enrolled in this study. After signing a written informed consent, cardiovascular risk factors and blood samples were collected following a 12-h fast. Sample were stored at -70˚C and analyzed for hemoglobin A1c (HbA1c) levels and lipid profiles, including serum low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (TGs), with the use of automated diagnostic equipment (DLS Laboratories). All participants underwent CAC scanning and CCTA to evaluate early CAD. Patients were randomized at a 1:1 ratio to receive 2,400 mg AGE/day or a placebo. The intended duration of administration for the study group was 12 months. This study was approved by the Institutional Review Board of Lundquist Institute for Biomedical Innovation at Harbor UCLA Medical Center (Clinicaltrials.gov: NCT03931434).

Inclusion criteria. Patients with T2DM were enrolled in this study. The age of the patients was between 30-75 years and the patients had HbA1c levels >6.5% or fasting blood sugar levels >125 mg/dl, or were taking anti-diabetic medications and who provided the written informed consent.

Exclusion criteria. Individuals with known hypersensitivity to AGE, a body weight >300 lbs, a history of bleeding disorders or those taking anti-coagulants, those with hypertensive encephalopathy or cerebrovascular event, a known history of CAD, MI, stroke or life-threatening arrhythmia within the prior 6 months, New York Heart Association Functional Classification II-IV heart failure, renal impairment (serum creatinine levels, >1.4 mg/dl), current tobacco use, or who were currently enrolled in another placebo-controlled trial or females with pregnancy were excluded.

Outcome measures. The outcome measures included the rate of change in coronary plaque volume and vulnerable component of coronary plaques.

AGE. AGE (Kyolic) is formulated by soaking sliced raw garlic in aqueous ethanol solution at room temperature for up to 20 months as previously described (11,12). The 2,400 mg of AGE capsules were provided by Wakunaga of America Co., Ltd. with a matched placebo pill. The placebo and the study drug appeared to be of similar size and color; however, the placebo pill did not contain any garlic or active ingredient. All participants were advised to take 2 capsules twice daily with water for 12 months. An inter-trial phone visit was conducted to ensure study medication compliance. The AGE capsule used in this study is commercially available in the market.

Data acquisition. The details of coronary artery plaque volume by CCTA assessment have been published in previous studies (6,15). Non-contrast scans for the evaluation of the extent of coronary artery calcium and contrast scans for the evaluation of coronary artery plaque volume at baseline and 12 months were acquired. Scans were assessed by readers who were blinded both to treatment group and to the date of the scan.

Coronary plaque assessment. Quantitative plaque assessment was performed according to a previously defined protocol (10) using semi-automated plaque analysis software (QAngioCT Research Edition Version 2.0.5; Medis Medical Imaging Systems). Based on the guidelines of the Society of Cardiovascular Computed Tomography, 17-segment coronary artery model vessels based on SCCT guidelines were assessed. Only vessels >1.5 mm were evaluated. The volumes of 4 types of coronary artery plaque [LAP, fibro-fatty (FF), fibrous (F) and dense calcium (DC)] were calculated using the Hounsfield unit threshold.

Statistical analyses. Continuous variables are expressed as the means ± standard deviation (SD), while categorical variables are stated as counts and percentages. A Student's t-test or Chi-square test was used to assess differences in all baseline parameters between the placebo and AGE. The analysis included comparisons for both plaque levels at both baseline and follow-up and between groups following the use of the ANOVA modeling with the Tukey approach P-value. ANCOVA modeling was also used to adjust for clinically significant variables. As none of these modeling techniques were significant, we thus chose to focus on the simple progression of LAP in the placebo group and regression in the active group. Atherosclerotic plaque volumes differences between the placebo and AGE were assessed by the Wilcoxon rank-sum test. A P-value <0.05 was considered to indicate a statistically significant difference. SAS software (version 9.4) was used to carry out all statistical analyses.

Results

From 2016 to 2017, 80 patients were enrolled and randomly assigned to the placebo or AGE groups. In total, 66 patients completed the study with baseline and follow-up CT. The participants had a mean follow-up period of 370±24 days. Of these patients, 28% in the placebo group and 29% in the active group were on statins. Patients in both groups continued to see their primary care physicians and continued their regular medications. All the baseline data at entry into this study in both groups have been previously described (16). No statistically significant differences were found between the groups.

The AGE group compared with the placebo group exhibited a statistically significant regression in normalized LAP [median SD) -0.2 (18.8) vs. 2.5 (69.3), P=0.0415]. By percentage, there was 29% reduction in LAP in the AGE group as opposed to 57% LAP plaque progression in the placebo group. The AGE group exhibited a reduction in FF plaque as compared

Table I. Quantitative plaque scores (median ± SD) in both groups.

Type of plaque	No.	Baseline (median ± SD)	P-value	Follow-up (median ± SD)	P-value	Difference with in the group (median ± SD)	Difference with in the group (Δ%)
DC			0.61		0.82		
Placebo	29	29.8±146.7		39.5±169		11.5±33.9	33%
Active	37	31.6±99.2		53.5±113.8		19.7±29.8	69%
FF			0.81		0.28		
Placebo	29	34.1±163.7		42.3±174.2		1.6±26	24%
Active	37	36.9±46.6		23.9±44.3		-0.1±23.5	-35%
F			0.64		0.60		
Placebo	29	148±288.7		163.4±281.7		14.3±136.6	10%
Active	37	148.5±193.2		186.3±188.3		21.6±84.5	25%
LAP			0.97		0.04		
Placebo	29	9.4±85.1		14.8±146.4		2.5±69.3	57%
Active	37	14±38.3		9.9±33.8		-0.2±18.8	**-29%**
TNCP			0.62		0.62		
Placebo	29	170.2±516.1		308.7±547.6		25.7±129.4	81%
Active	37	220.2±243.6		283.4±243.4		26.5±96.7	29%
TPV			0.79		0.50		
Placebo	29	223.9±595.2		347.9±657.9		53.3±141.2	55%
Active	37	275±316.7		328.4±313.1		38.7±100.9	19%

Values are the means ± SD. Values in bold font indicate statistically significant differences (P<0.05). DC, dense calcium; FF, fibro-fatty; F, Fibrous; LAP, low-attenuation plaque; TNCP, total noncalcified plaque; TPV, total plaque volume.

to the progression of FF in the placebo group; however this did not reach statistically significance (median difference -0.1 vs. +1.6, P-0.287). No marked differences were observed in total plaque (TP), F, or FF plaque volumes between the AGE and placebo group (Table I and Fig. 1).

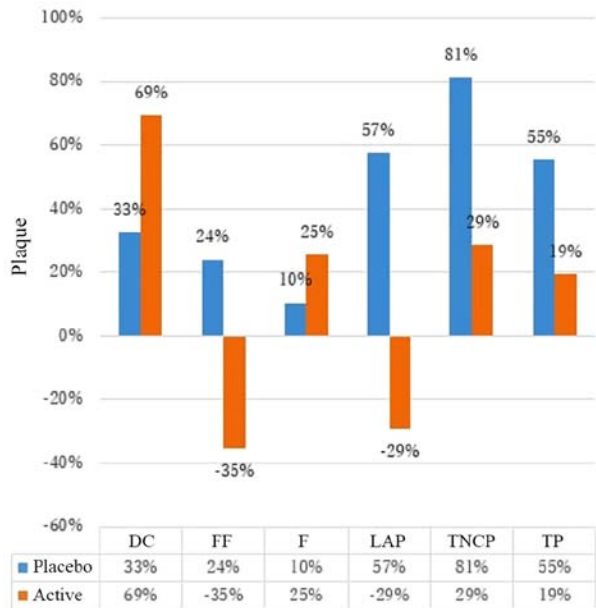

Figure 1. Plaque differences in both groups at 12 months. Percentages (%) of coronary atherosclerotic plaque volume changes of dense calcified (DC), fibrous fatty (FF), fibrous (F), Low attenuation plaque (LAP), total non-calcified plaque (TNCP) and total plaque (TP)among both the groups at 12 months.

Discussion

In this study, 1 year of AGE therapy was associated with a regression of LAP. LAP [non-calcified plaque (NCP) <30 HU] is one of the high-risk plaque features. The results of this study are in concordance with those of previously published studies demonstrating the regression of LAP in participants with metabolic syndrome and general population in studies of AGE (10,17), as well as statin studies (18,19).

According to certain data, LAP represents an intravascular ultrasound (IVUS) equivalent of the necrotic core (19). In contrast to more stable F plaques, plaques with a lipid-rich necrotic core play a significant role in the development of acute coronary syndrome (ACS) (20). Recent advancements in cardiac imaging modalities have provided an accurate and detailed quantitative assessment of plaque composition and burden based on differences in CCTA (21). Previous studies have demonstrated an increased risk of myocardial infarction and cardiovascular death in patients with a larger volume of non-calcified and low-attenuation plaque (22,23).

Motoyama *et al* (24) demonstrated that patients with ACS exhibited a significantly increased frequency of low attenuation plaque (79 vs. 9%, P<0.001). Balestrieri *et al* (9) reported that the patients with stable chest pain with adverse plaque features, including LAP, spotty calcification and positive remodeling found an association with a 60% increased risk of future adverse cardiovascular events. Furthermore, LAP along with other vulnerable plaque features have been shown to be associated with rapidly progressive CAD (25). The SCOT-HEART trial, which consisted of mostly intermediate risk patients, demonstrated that low attenuation was associated with a 3-fold increased risk of non-fatal MI or coronary heart disease-associated mortality (26). In this study, there was a 57% increase in low attenuation plaque in the placebo group, representing the natural history of plaque progression. By contrast, a 29% reduction was observed in LAP in the AGE group over a period of 1-year follow-up, strongly suggesting the therapeutic potential of AGE in the diabetic population (Fig. 1).

The present study has several limitations. First, this relatively small sample size and short-term follow-up study did not have enough power to demonstrate the significant differences in TP volume, NCP and DC. Second, patients were under different therapies for hyperlipidemia and T2DM, and some patients used varying medications at different doses. Due to the small sample size, a separate analysis with different hyperlipidemia and T2DM medications was not performed. Further research is thus required to evaluate whether AGE has the ability to stabilize vulnerable plaque and to decrease adverse cardiovascular events.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

Authors' contributions

MJB conceived of and designed the study. KS, LC, DB, RN, SA, EJ, CS, FF, SH, MSH, AJ, BC and MJB collected the patient information and generated the clinical data. AK, KS, LC, DB, RN, SA and MJB analyzed and/or interpreted the data; and KS, AK, LC, RN and MJB drafted or revised the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Lundquist Institute for Biomedical Innovation at Harbor UCLA Medical Center (Clinicaltrials.gov: NCT03931434). All patients provided signed and written informed consent.

Patient consent for publication

Not applicable.

Competing interests

MJB discloses work for the National Institutes of Health and General Electric Healthcare. All the other authors declare that they have no competing interests.

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